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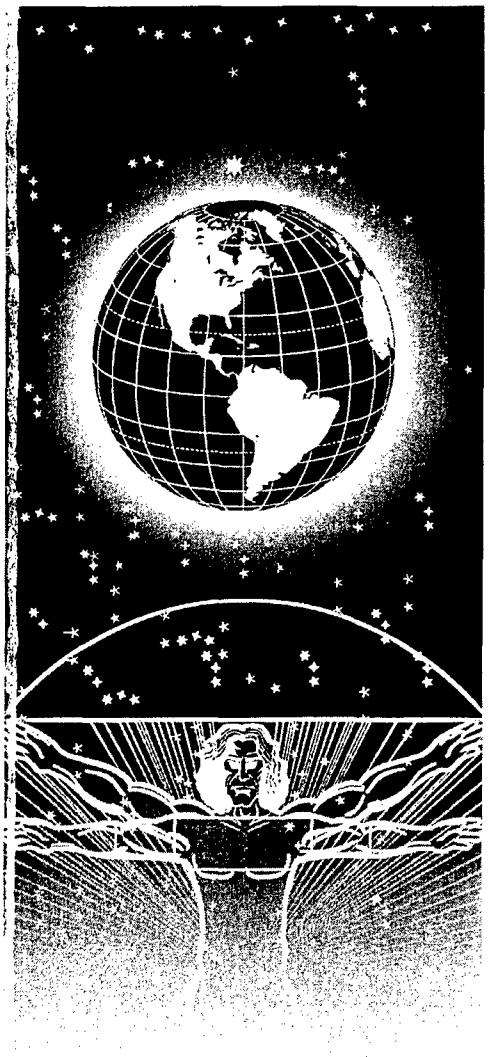

UNITED STATES AIR FORCE RESEARCH LABORATORY

IMMUNOTOXICITY OF JET FUELS AND SOLVENTS

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

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FOR THE DIRECTOR

Mark M. Hoffman

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Immunotoxicity involves an adverse immune response to allergens, chemicals or other xenobiotics. Chemicals can affect a number of the complex components of the immune system. Changes in the immune system as the result of chemical exposure to jet fuels or other solvents are a concern for the occupational worker. This report reviewed studies involving the effects of jet fuels on the immune system; components of jet fuel or solvents with known immunotoxicity were also discussed. Personnel working with military and commercial fuels such as hydrazine, JP-8 and Jet A could be at risk for immunosuppression as this is a major effect of these fuels.

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PREFACE

This review was conducted at the Air Force Research Laboratory, Human Effectiveness Directorate, Operational Toxicology Branch (AFRL/HEST) at Wright-Patterson Air Force Base, OH. The work was performed under the following supervision and Project/Work Unit: Branch Chief: Dr. Richard Stotts (AFRL/HEST); Project Director: David R. Mattie (AFRL/HEST); and Work Unit: 1710D432, Toxicity Assessment/Methods Development for Weapon Life Cycle Costs.

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LIST OF ABBREVIATIONS

CCl_4	carbon tetrachloride
$CD3^+$, $CD4^+$, $CD5^+$	T-cells expressing CD3, CD4 or CD5 accessory molecules
Con A	Concanavalin A
CP	cyclophosphamide
DTH	delayed-type hypersensitivity
EPA	Environmental Protection Agency
GSH	glutathione
hr	hour
IgE, IgG, IgM	immunoglobin isomers
IL-2	interleukin 2
ip	intraperitoneal
KLH	keyhole limpet hemocyanin
m^3	cubic meter
mg	milligram
NIOSH	National Institute of Occupational Safety and Health
$O_2^{\bullet}$-	superoxide anion
OEL	Occupational Exposure Level
OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
pan-T	all T-cells
PFC	plaque forming cells
PFDA	perfluorodecanoic acid
PGE_2	prostaglandin E_2
PMC	diphenyl dimethyl dicarboxylate
ppm	part per million
SM	2,2'-dichlorodiethyl sulfide
SP	substance P
sRBC	sheep red blood cell
TCE	trichloroethylene
TDI	toluene diisocyanate
T_H	T helper cell
TLV	Threshold Limit Value

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IMMUNOTOXICITY OF JET FUELS AND SOLVENTS

INTRODUCTION

Immunotoxicity reflects adverse immune responses to allergens, chemicals or other xenobiotics. On one side, immunostimulating drugs are a course of treatment for diseases, such as cancer (Sharma, 1984), while other compounds, such as corticosteroids, suppress immune function or response. There is a delicate balance between immune suppression and immune enhancement, as demonstrated by studies with tumor immunotherapy (Kuby, 1992). Since the immune system is incredibly complex, it is difficult to discern the capability of industrial chemicals to affect immune function. For example, using cell-mediated and antibody-mediated (humoral) responses, it is feasible that the immune system could react immediately to chemical exposure by allergic hypersensitivity. The immune response to that same chemical could be changed if exposed to another xenobiotic that alters stimulation of antigens, resulting in immunomodulation (Sharma, 1984). This raises important questions about immunomodulation of workers exposed to multiple chemical agents with potential effects on the immune system.

This report focuses on information pertaining to changes in the immune system as the result of chemical exposure by the occupational worker to jet fuels or other solvents. Although this review doesn't attempt to answer this question, it is important know whether current Occupational Exposure Levels (OELs) and Threshold Limit Values (TLVs) established by the Occupational Safety and Health Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH), respectively, provide accurate information about levels of protection with agents that cause changes in immunity. Future risk assessments need to address immune stimulating or depressing chemicals.

Research indicates that the general public could be in danger of immune effects from environmental chemical exposure. Patients using immunosuppressive medications for extended periods of time or people exposed to even low levels of environmental chemicals may be unable to fight infections and tumors, may become hypersensitive to chemicals, or develop autoimmune diseases. The immune system is able to respond to environmental chemicals at extremely low levels (Luster and Rosenthal, 1993). If these chemicals are present in the environment through natural occurrence or via contamination, there could be immunological changes in the general population that contribute to disease (Luster and Rosenthal, 1993). Dourson and Younes (2001) reported on risk assessment with respect to immunotoxicity and demonstrated some of the complicating factors between different regulatory bodies, a lack of standard protocols and the variety of species-specific results. There does not seem to be a good correlation between the probabilities of specific, predictable immune responses with exposure to a particular chemical agent.

EFFECTS OF TOXICANTS ON THE IMMUNE SYSTEM

Through extremely complex yet specific mechanisms, profiles of immune system modulation can reflect apparent signs of immunotoxicity and/or immunotoxicological responses. Although the immune system predominantly functions through the action of innate or antigen-specific cell-mediated and antibody (humoral) responses, there are soluble effector molecules that can reflect immunotoxicological activation. Moreover, the effect of toxic agents on the immune system can ultimately lead to immunosuppression, thus attenuating the capability of resolving infection or immunocompromise in a timely manner.

Cell-mediated and humoral immune responses are two distinct systems that work together to affect the overall immune response (Sharma, 1984). Cell-mediated responses include innate and antigen-specific mechanisms. Innate responses include phagocytic cells, such as neutrophils and macrophages, as well as complement factors. Antigen-specific cell-mediated responses are derived from the activation of T cells following presentation of antigens by professional antigen-presenting cells. Activated T cells can help modulate the T-helper 1 (T_H1) mechanisms, which mainly utilize direct action of T cells to resolve bacterial/viral infections. The T_H2 response works to stimulate the antibody response. Once B cells have encountered a foreign antigen, they are stimulated to produce antibodies to neutralize the specific antigen. These B cells can then differentiate into antigen-specific memory cells or plasma cells. T_H2 cells play a role in the antibody response by releasing specific cytokine profiles that stimulate memory B cells or plasma cells to produce specific antibody isotypes (e.g., IgG, IgA, etc). T cells can also act in delayed-type hypersensitivity (DTH) reactions, which can reflect antigen- or non-antigen-specific allergic responses.

Toxicological stimuli can act as perturbations affecting full development of an immune response. An example is the reduced production of interleukin 2 (IL-2) by mitogen- or antigen-activated T_H1 cells (Reid *et al.*, 1994). IL-2 is a significant factor in production and proliferation of T-cells, triggering and managing the immune response (Reid *et al.*, 1994; Kuby, 1992). T_H1 cells can to produce IL-2 within 24 to 48 hours after activation by an antigen or mitogen (Kuby, 1992). Mice given an intraperitoneal injection of benzene or cyclosporin A demonstrated that production of IL-2 was markedly decreased by both agents, suggesting an immunosuppressive effect (Reid *et al.*, 1994). Blank *et al.* (1991) published research comparing the immunotoxicity of two mustard agents, 2,2'-dichlorodiethyl sulfide (SM) and cyclophosphamide (CP), via cell-mediated immunity, humoral immunity and tumor cell resistance. Cell-mediated immune function in mice was evaluated using the delayed type hypersensitivity (DTH) response to keyhole limpet hemocyanin (KLH); no changes in delayed hypersensitivity response were seen in SM and CP-exposed mice. Humoral response, in the form of the IgM antibody response, revealed considerable immune suppression. Significantly suppressed host resistance was also noted when CP-dosed animals were challenged with L1210 tumor cells (Blank *et al.*, 1991).

IMMUNOTOXICITY STUDIES – JET FUELS

Jet fuel is an environmental chemical to which exposure results in significant immunotoxic effects. The jet fuel JP-8 is comprised of a mixture of aliphatic and aromatic hydrocarbons, with

approximately 81% alkanes, 10 to 20% polycyclic aromatic hydrocarbons and trace amounts of benzene, xylene and toluene. Several individual components of the fuel, such as benzene, are known to be immunotoxic and could therefore contribute to the overall immune reaction (Dudley *et al.*, 2001). The average concentration of benzene in JP-8 was determined to be 270 ppm (Mayfield and Howard, 1996). Even inhalation exposure to low concentrations of aerosolized military jet fuel JP-8 (100 mg/m^3) for short time periods (1 hr/day for 7 days) resulted in long-term immunosuppressive effects in mice, shown as decreased spleen and thymus weights, reduced cell numbers and overall depressed immune response using mitogenic assays (Harris *et al.*, 1997a). At 1000 mg/m^3 exposures for the same time frame, immune response in mice was depressed for 4 weeks after JP-8 exposure. These long-lasting effects could pose significant problems for individuals exposed to concentrations of JP-8 at or greater than 100 mg/m^3. Long-term immunosuppression such as this reduces the host defense system's ability to fight infectious disease, carcinogenic tumors, chemical hypersensitivity and autoimmune diseases. Furthermore, continuous, recurring exposures rob the immune system of time it needs to recover, potentially causing even more damage (Harris *et al.*, 1997b). Differing in their additives, JP-8+100 and commercially used Jet A1 exhibited the same results with decreased organ weights and reduced thymus and spleen cell numbers in mice exposed to inhalation exposures of aerosolized jet fuel (Harris *et al.*, 2000).

Dermal exposures to jet fuels are also a concern for immunosuppression, since skin contact is frequent during operations like refueling and fuel tank entries. Since JP-8 has a lower vapor pressure than its predecessor JP-4, it tends to absorb into skin rather than evaporate (Ramos *et al.*, 2002). Skin exposure to JP-8 and Jet-A in mice caused immune suppression, measured by decreases in the elicitation of classic DTH, suppression of contact hypersensitivity induction and decreased T-cell proliferation (Ullrich and Lyons, 2000; Ramos *et al.*, 2002). Humoral immune function was evaluated by antibody response to KLH in JP-8 exposed mice and found to be less vulnerable to JP-8-mediated immune suppression than cell-mediated immune responses, possibly because JP-8 targets T helper-1 cell function and not T helper-2 cells (Ullrich and Lyons, 2000). Concern lies in the realization that jet fuels not only affect immune response, but immune memory as well, demonstrated by DTH to the fungus *Candida albicans*. However, a selective cyclooxygenase-2 inhibitor, SC 236, prohibits prostaglandin E_2 (PGE_2) production and blocks immune suppression. This could allow exposed personnel, military and civilian, to counteract jet fuel-induced immune effects with a cyclooxygenase inhibitor (Ramos *et al.*, 2002).

Harris *et al.* (1997c; 2000) also looked at prophylactic protection from jet fuel exposures, specifically inhalation exposures to JP-8. Their studies focused on the neuropeptide substance P (SP), which was shown to protect against changes in pulmonary function from JP-8. JP-8-exposed mice given aerosolized SP after exposure had increased spleen and thymus cell numbers and normalized responses to Concanavalin A (Con A) and IL-2 stimulation (Harris *et al.*, 1997c). Pre-exposure SP administration showed varying prophylactic measures, from prevention of cell loss and organ weight loss with one and six hour pretreatments, to prevention of immune dysfunction with a 15 minute pre-exposure (Harris *et al.*, 2000). It is possible that SP could be used both before and after JP-8 inhalation exposures to protect workers from short and long-term immunosuppressive effects.

Hydrazine, specifically 1,1-dimethylhydrazine, and hydrazine-based fuels are a form of liquid propellant used in rockets and emergency power units for military aircraft (Grove, 1998). Experiments with 1,1-dimethylhydrazine have yielded inhibited Con A-stimulated DNA synthesis and suppressed IL-2 production, immune effects consistent with JP-8-induced immunosuppression. These authors also noted that hydrazine has been linked to cases of the autoimmune disease, systemic lupus erythematosus, possibly because problems with IL-2 are connected to immune deficiency (Bauer *et al.*, 1990).

Personnel working with military and commercial fuels like hydrazine, JP-8 and Jet A could be at risk for delayed immunosuppression, even at relatively low exposures. This prolonged depression of the immune defenses could increase changes of infectious and autoimmune diseases, and would be compounded if personnel were exposed to multiple chemicals, physical labor and stressful situations, as would aircraft mechanics or other flightline personnel. Add the additional physical and mental stressors of a deployed location for a military member and immunotoxicity becomes an incredibly important question for the health of military personnel.

IMMUNOTOXICITY STUDIES – SOLVENTS

Benzene

Benzene has already been mentioned as an important immune suppressing chemical component of JP-8. It has been used as an industrial solvent, and is frequently seen in environmental contamination. Benzene exposure has been linked to hematotoxicity, hematopoietic dysfunction (Robinson *et al.*, 1997), bone marrow stem cell damage and pancytopenia (Wierda *et al.*, 1981; Bogadi-Sare *et al.*, 2000). Lymphocytes, specifically precursor lymphocytes, are the most susceptible targets for benzene and its metabolites, such as catechol, hydroquinone, 1,2,4-benzenetriol and benzoquinone. Catechol has been shown to damage B cell production by causing lymphocyte death or by preventing precursor cells from maturing, while the other metabolites have been implicated in bone marrow and splenic lymphocyte degradation (Wierda *et al.*, 1981).

Rats exposed to inhaled benzene at 400 ppm for 4 weeks (6 hr/day, 5 days/week) showed spleen and thymus weight reduction and reduced spleen cell numbers in $Kappa^+$ (B-) lymphocytes, $CD4^+/CD5^+$ (T_H) cells, and $CD5^+$ (pan-T) lymphocytes (Robinson *et al.*, 1997). A human study found significant B lymphocyte depression in workers exposed to concentrations of 15 ppm or less benzene in solvents compared to a control population (Boagdi-Sare *et al.*, 2000). The human data are comparable to animal studies documenting compromised T- and B-lymphocyte function after exposure to benzene (Wierda *et al.*, 1981; Robinson *et al.*, 1997). McMurry *et al.* (1994) published results in which rats ip injected with benzene did not exhibit T- and B-cell dysfunction, possibly because the animals were sacrificed nine days after the last exposure, giving time for the benzene to metabolize and for recovery, whereas the Robinson *et al.* (1997) study euthanized rats within three hours after the final exposure. Selective B lymphocyte suppression with benzene exposures can also possibly due to the short life span of B cells (Aoyama, 1986). Findings were different in an intraperitoneal administration study that showed

no distinction in the suppression of T and B lymphocytes (Wierda *et al.*, 1981). Discrepancies could be the result of dissimilar exposure methods or doses (Aoyama, 1986). Oral nitrobenzene exposures caused suppression of murine spleen cell response to T-cell mitogen, but no adverse effect to B cell mitogen (Burns *et al.*, 1994a).

Pretreatment with Aroclor 1254 reduced splenic cell toxicity in mice later injected with benzene (Wierda *et al.*, 1981). Pretreatment allowed increased mitogen-stimulated lymphocyte response, but provided no protection from PFC depression, indicating that the Arochlor may compete metabolically with benzene and that the benzene immunotoxic effects may likely stem from metabolites and not the parent compound (Wierda *et al.*, 1981).

Toluene/Toluene Mixtures

Hsieh *et al.* (1990) conducted a study on combined benzene and toluene oral exposures in mice and reported that toluene had a protective effect on α-sRBC antibody levels and PFC numbers compared to benzene exposures alone. Benzene exposures in this study produced decreased lymphocytes, leukocytes and total blood erythrocytes. A hypothesis for the protective effect of toluene against benzene is that toluene, as a substrate for cytochrome P450, competitively inhibits biotransformation of benzene into its metabolites, which are responsible for immunotoxicity. Another proposal is that toluene increases detoxification reactions in the liver, decreasing the amounts of reactive benzene oxidative metabolites.

The effects of an inhaled 1:1 toluene/n-hexane mixture produced immune suppression results of decreased host resistance to *Mycobacterium bovis*, weight loss and physical changes in the liver, lung and spleen. Increased cortisol levels indicate exposed hamsters were more stressed than control groups and had significantly greater colony-forming units of *M. bovis* located throughout liver, lung and spleen (Palermo-Neto *et al.*, 2001).

Burns *et al.* (1994b,c) studied the oral effects of para- and meta-nitrotoluene in mice, reporting humoral and cell-mediated immune effects. Both studies showed modest dose-dependent weight gains, increased liver weight, reversible hepatocyte swelling, decreased $CD3^+/CD4^+$ T-cells, decreased IgM antibody-forming cell response to sRBC and decreased resistance to *Listeria monocytogenes*. p-Nitrotoluene exposures produced an impaired elicitation of DTH response, a T-cell dependent assay, consistent with the authors' hypothesis that p-nitrotoluene affects cytokine release, production or signaling. The lack of host resistance to *L. monocytogenes* also proved a decreased T-cell mediated immune response, possibly related to decreased numbers of splenic $CD4^+$ T_H cells (Burns *et al.*, 1994c). Compared to p-nitrotoluene and m-nitrotoluene, toluene does not appear to target the immune system. Toluene did not affect splenic T-cells and the IgM response to sRBC, which were inhibited by both p- and m-nitrotoluene (Burns *et al.*, 1994b,c). Increased natural killer cell activity was noted with m-nitrotoluene exposure but decreased with toluene. These results indicate that isomers of nitrotoluene are metabolized differently than the solvent toluene and affect systems differently (Burns *et al.*, 1994c).

Carbon Tetrachloride

Carbon tetrachloride (CCl_4) was used in a variety of cleaning products and solvents before concern over its toxicity caused production to decrease in the late 1980s (Smialowicz *et al.*, 1991). Like benzene, carbon tetrachloride is hydroxylated in the first step of chemical breakdown into hematotoxic (benzene) and hepatotoxic (CCl_4) metabolites by cytochrome P450, part of a family of oxidative detoxification enzymes (Daiker *et al.*, 2000; Smialowicz *et al.*, 1991; Voet *et al.*, 1999). Oral dosing in rats showed no effects on humoral immunity, as measured by the PFC response to sRBC, though dose-dependent hepatotoxicity was noted (Smialowicz *et al.*, 1991). Kaminiski *et al.* (1992) also credited an inhibited IgM antibody forming cell response in mice experiments to the cytotoxic effects of CCl_4. *In vitro* studies with spleen and liver cells demonstrated again that CCl_4 requires metabolic bioactivation but also that it inhibits T-cell dependent antibody response, not affecting T-cell independent antibody response. These findings suggest that a metabolic intermediate of CCl_4 is responsible for its immunotoxic effects (Kaminiski *et al.*, 1992).

Prophylactic use of chemicals such as neuropeptide SP and selective cyclooxygenase-2 inhibitor SC 236 for protection against JP-8 have been discussed and provide hope for continued studies of immunoprotective possibilities. Ahn and Kim (1993) documented the protective effects of diphenyl dimethyl dicarboxylate (PMC) against CCl_4 in orally dosed mice. This study did find marked suppression of humoral and cell-mediated immune response to oral dosing, but PMC proved effective in reversing those effects. Body, spleen and thymus weights all increased after being depressed from CCl_4 exposure. Hemagglutination titers were restored after treatment with PMC, showing a return in antibody response. PMC also restored humoral immunity to the T-dependent antigen sRBC, suppressed the elevated delayed-type hypersensitivity response to CCl_4, increased depressed natural killer cell activity and restored phagocyte activity and leukocyte numbers (Ahn and Kim, 1993).

Trichloroethylene

Trichloroethylene (TCE) is a common industrial chemical used in cleaners and degreasers (Kaneko *et al.*, 2000) and was used medically as a general anesthetic (Barton and Clewell, 2000). Kaneko *et al.* (2000) reported dose-dependent decreased murine serum IgG concentrations and histopathological changes to the liver and spleen with TCE exposures. These effects are reportedly found more frequently in female mice, suggesting they are more sensitive to the chemical; however males metabolize more TCE (Kaneko *et al.*, 2000; Barton and Clewell, 2000). It must be taken into consideration when discussing murine exposure data to TCE that mice metabolize TCE more effectively than humans or rats. In fact, TCE has a longer residual in the human body than rodents because of the recirculation of its metabolites. Because of these complexities, alternative methods have been investigated using oral studies to develop reference doses and inhalation studies to develop reference concentrations for human exposures to TCE (Barton and Clewell, 1998).

Metabolites - Quinones

Quinones are defined as a "class of toxicological intermediates" that act upon a living system to produce a host of toxic effects on individual cells and the immune system. They can cause cellular damage by covalently binding with proteins and DNA and are also highly reactive compounds capable of forming reactive oxygen species. Quinones can therefore cause a wide variety of deleterious effects through alkylation or oxidative stress pathways (Bolton *et al.*, 2000).

Hydroquinone is one of the metabolites of benzene that is formed by oxidative metabolism and exerts its hematotoxicity by collecting in the bone marrow and inhibiting hematopoiesis. King *et al.* (1987) reported that acute, low dose *in vitro* exposure to hydroquinone adversely affects the maturation of precursor B cells. Hydroquinone has also been shown to prevent transcription factor nuclear factor-κB activition, effectively inhibiting T-cell function (Pyatt *et al.*, 1998) and affecting humoral immunity and B cell production. Unlike the reversibility of T-cells to respond to damage, hydroquinone-induced B cell inhibition does not rebound. This results in suppression of B cell maturation, differentiation and overall production (Pyatt *et al.*, 2000).

Another metabolite, ortho-quinone, is derived from polycyclic hydrocarbons (PAHs) and alkylates cellular nucleophiles. o-Quinone has been shown to be cytotoxic to human and rat liver cells, causing cell death via radical-mediated cell damage or glutathione (GSH) depletion, and is mutagenic. Oxygen radicals have been implicated in diseases from breast cancer to Parkinson's disease. Reactive oxygen species are responsible for damage to DNA, causing chromosomal abnormalities and single-strand breaks, and can activate signaling pathways, causing even more destruction (Bolton *et al.*, 1999). Metabolism of xenobiotics to quinones could contribute to genetically based disease, cancers and cytotoxicity, making exposures to environmental toxins even more dangerous.

Other Solvents

Tarr and Mathes (1991) evaluated the peroxisome proliferator perfluorodecanoic acid (PFDA) for its hepatotoxic and immunotoxic effects in several studies looking at humoral and cell-mediated immune response. The proliferation of lymphocytes to the T-cell mitogen Con A was reduced with PFDA exposure, while lymphoproliferation to the B cell mitogen lipopolysaccharide or KLH antigen increased. Natural killer cell activity was reportedly increased 30 days after PFDA exposure.

Pulmonary hypersensitivity from aerosol isocyanate exposure is well documented and known throughout the occupational health world. Of particular concern is exposure to toluene diisocyanate (TDI), and its common 2,4- and 2,6- isomers used in industry, both of which have been shown to elicit an immune response in guinea pig exposures. Humans exposed to isocyanate with resulting respiratory symptoms typically have isocyanate-specific IgE antibodies in their systems, which could potentially be used to estimate isocyanate exposures in an industrial setting (Karol and Jin, 1991). Fowle and Sexton (1992) reported that the U.S. Environmental Protection Agency (EPA), responsible for protecting human health from environmental exposures, is relying more heavily on biologic markers to determine health risk

assessments for regulatory decision making. These biologic measurements were described as part of the "state-of-the-art" science in developing health risk management plans for the incredible assortment of environmental toxins to which humans can be exposed (Fowle and Sexton, 1992). The human IgE antibody results and animal studies seem to show dose-dependent antibody production, a potential biomarker of occupational isocyanate exposure (Karol and Jin, 1991).

SUMMARY

The immune system can serve as a sensitive indicator for a wide variety of chemical exposures. Though the pathways of chemical exposure to immune response are complex and not well elucidated, one can be sure that many chemicals are immunotoxic or produce immunotoxic effects on the humoral and/or cell-mediated immune system. Adverse immune responses that define immunotoxicity show up in many target cells and organs. Lymphoid organs, kidney, liver, spleen and thymus, often show immunotoxic effects by weight loss and/or morphological changes. Exposures to jet fuels (Harris *et al.*, 1997a), benzene (Robinson *et al.*, 1997) and carbon tetrachloride (Ahn and Kim, 1993) all resulted in decreased spleen and thymus weights. Histopathological changes to the liver and spleen were noted with TCE exposures (Kaneko *et al.*, 2000). Toluene exposures also caused physical changes in the liver, lung and spleen (Palermo-Neto *et al.*, 2001).

The humoral immune response to these chemicals was often tested with the IgM antibody response to the T-dependent antigen sRBC by the formation of antibody PFC in the spleen and challenges with tumor cells. Cell-mediated immunity relied on mitogen-stimulated T-cell lymphocyte proliferation, IL-2 production and DTH response to KLH or sRBC.

In the testing of fuels, immune suppression was quantified with cell proliferation assays, showing a significant decrease in immune response and overall cell numbers over an extended time (Harris *et al.*, 1997a,b). Immune memory was also severely affected with fuel exposure and *C. albicans* challenge (Ramos *et al.*, 2002). In dermal exposures, humoral immune function was less vulnerable to JP-8-mediated immune suppression than cell-mediated immune responses (Ullrich and Lyons, 2000). Hydrazine showed effects similar to JP-8 with IL-2 production and suppressed DNA synthesis (Bauer *et al.*, 1990).

Benzene exposures yielded B and T lymphocyte depression (Wierda *et al.*, 1981; Robinson *et al.*, 1997), though other studies had conflicting results, possibly due to different methods or doses (Aoyama, 1986). The quinone metabolites of benzene showed definite T and B cell inhibition (Pyatt *et al.*, 2000). Carbon tetrachloride also had differing results with one study demonstrating no suppression of humoral immunity (Smialowicz *et al.*, 1991) and another with significant suppression of both humoral and cell-mediated immune response (Ahn and Kim, 1993). Trichloroethylene produced dose-dependent decreased murine serum IgG concentrations (Kaneko *et al.*, 2000). The isomers of nitrotoluene showed substantial humoral and cell-mediated immune suppression, but toluene itself did not appear to target the immune system (Burns *et al.*, 1994b,c). In fact, toluene exposures combined with benzene partially blocked the immunosuppressive effects of benzene alone (Hsieh *et al.*, 1990).

There are issues about immune memory and how exposure to immunosuppressive chemicals could affect the ability to fight disease. Environmental exposure to these chemicals warrants discussion about potential immune problems in the public and contribution to infectious disease and cancers (Luster and Rosenthal, 1993). Probably the most poignant fact is that the immune system is indeed incredibly sensitive and responds to chemical exposure at extremely low levels (Zelikoff *et al.*, 2000). If continuing studies can elucidate specific chemical-induced immune system mechanisms and translate these biomarkers into exposure data, prophylactic or therapeutic treatment against the effects and repercussions of immunotoxic chemical exposures could be implemented in the future. This could ultimately lead to linking changes in and the effects of the immune system in occupational health analysis.

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